A particular advantage of the method of the invention is the possibility of varying the range and slope of the pH gradient simply by varying the temperatures of the fluids flowing through the chambers **18** and **20**. There is actually no lower limit to the shallowness of the pH gradient which may be produced to enhance the resolving power of the system.

It may be pointed out that in order to separate a given pair of ampholytes, it is not necessary for the pH gradient to contain points of a pH equalling their pI values. It is merely necessary for it to contain a point of pH intermediate the two pI values. In such a case the two proteins will migrate in opposite directions toward the upper and lower boundaries of the M region where they will be condensed into sharp zones by conductivity gradient focusing, such focusing being described, for example, in an article by A. Kolin, J. Chem. Phys. **23**, 407 (1955).

It is obvious that different types of buffer solutions may be used in the practice of the method of the invention, and that materials other than sucrose may be used to increase the density of the buffer solution so as to stabilize against thermal convection. Moreover, such stabilization against thermal convection may be achieved without density gradients by providing a conductivity gradient in a magnetic field, by rotation of the U-tube **10** about a horizontal axis, by means of porous media such as filter paper or other stabilizing matrices, laminar flow through a thin liquid curtain, and the like.

Also, it is evident that the temperature differential within the electrophoretic column may be established by means other than those illustrated in the drawing. For example, the electric current in the electrolyte may be used to establish a desired temperature gradient. When the electric current is used to establish the temperature gradient, the U-tube may have a trapezoidal or other selected configuration, so that the resulting heat generated by the electric current flow may be created with any desired temperature gradient, both uniform or non-uniform. The temperature gradient, moreover, may be created in discrete steps, or in discrete compartments, so as to as to achieve any desired resolution in the resulting isoelectric spectrum, and in selected parts of the spectrum. The container **10** need not ncessarily be a U-tube, and may be any type of vessel, cell, column, container, tube, or the like.

It may also be pointed out that the temperature differential created in the apparatus need not necessarily create a corresponding pH gradient in the buffer solution, since with various ampholyte mixtures, the pI of the various ampholytes may be temperature dependent. In that case, the buffer pH may remain constant, whereas the pI of the various ampholyte fractions may be controlled so as to distribute the resulting isoelectric points through the M region in the drawing. Specifically, therefore, the temperature gradient may be established to control either the pH of the buffer solution, or the pI of the ampholyte, or both. Moreover, the temperature differential may be used, in some instances, to establish a gradient in the (pH – pI) values of the ampholyte and buffer solution within the system.

It follows, therefore, that although a particular embodiment of the process of the invention has been shown and described, modifications may be made, and it is intended to cover all such modifications in the following claims.

What is claimed is:

1. A process for redistributing ampholytes, or the like, in an electrolytic buffer solution which comprises:

producing an electric current flow in a buffer solution containing at least one ampholyte in a direction to cause ions of the ampholyte to migrate electrophoretically in directions predetermined by factors including their isoelectric pH; and

establishing, in the buffered solution, a temperature gradient having a significant component substantially aligned with said current flow,

whereby the ions are redistributed in the buffered solution, and isoelectric focusing of said ampholyte takes place in the buffer solution.

2. The process defined in claim **1**, in which the result of said process is a separation of said ampholyte from other components in the buffer solution, and including the further step of recovering said ampholyte.

3. The process defined in claim **2**, and which includes the step of collecting the ampholyte at the isoelectric point thereof in said buffer solution.

4. The process defined in claim **1**, in which said ampholyte is concentrated in the buffer solution.

5. The process defined in claim **1**, in which said temperature gradient establishes a pH gradient in the buffer solution.

6. The process defined in claim **1**, in which said temperature gradient establishes a gradient in the isoelectric pH of the ampholyte.

7. The process defined in claim **1**, in which said temperature gradient establishes a gradient in the difference between the pH of the buffer solution and the isoelectric pH of the ampholyte.

8. The process defined in claim **1**, in which said temperature gradient is established by circulating fluids of different temperatures in thermal contact with the buffer solution.

9. The process defined in claim **1**, in which said buffered solution contains a plurality of ampholytes, and

said temperature gradient produces an isoelectric spectrum of the ampholytes in the buffer solution.

10. The process defined in claim **7** which includes the step of collecting the respective ampholytes individually at their respective isoelectric points in said buffered solution.

11. The process defined in claim **1**, in which said buffer solution is stabilized against thermal convection currents.

12. The process defined in claim **1**, in which said temperature gradient is produced in discrete steps.

13. The process defined in claim **1**, in which said buffer solution is stabilized by a density gradient of a selected solute.

14. The process defined in claim **1**, in which said buffer solution is introduced into a vessel of a selected configuration, and in which said electric current is provided by electrodes at the respective ends of said vessel.

15. The process defined in claim **14**, including the step of creating said temperature gradient by circulating, in thermal contact with said vessel, a fluid having a temperature significantly different from that of said buffer solution.

16. The process defined in claim **14** including the steps of

creating said temperature gradient by circulating, in thermal contact with said vessel, a first fluid having a given temperature and

a second fluid having a temperature differing significantly from that of first fluid,

said thermal contact being at locations spaced along the direction of said current flow.

17. The process defined in claim **1**, in which the migration of said ampholyte ions results in redistribution of the ampholyte, so that said ampholyte can be characterized by its isoelectric pH.

18. The process defined in claim **17**, in which the characterization of said ampholyte by its isoelectric pH is effected thermometrically.

19. The process defined in claim **1**, in which said temperature gradient is produced by generating heat within the buffered solution.

17. The process defined in claim **1**, in which the migration of said ampholyte ions results in redistribution of the ampholyte, so that said ampholyte can be characterized by its isoelectric pH.

18. The process defined in claim **17**, in which the characterization of said ampholyte by its isoelectric pH is effected thermomentrically.

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